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Original Article

Effects of three anesthetics of clove extract, sodium bicarbonate, and lidocaine on blood parameters and cortisol level on male and female broodstocks of Caspian kutum (*Rutilus kutum*)

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Abstract:
In this study, 32 male and female Caspian kutum were anesthetized with clove extract (50 mg/L), lidocaine (150 mg/L), and sodium bicarbonate (300 mg/L). Blood parameters and serum cortisol levels were investigated in the control (no anesthetic) and the treated groups containing both males and females in 10 min and 24 h after anesthesia. Based on the result, difference in the red blood cells in the control group and treated groups males was not significant. In females, however, the red blood cells in all treated groups was different except lidocaine treatment after both sampling times (P<0.05). The white blood cells of males and females in control group was significantly different in the sodium bicarbonate group in 10 min and 24 hours after anesthesia (P<0.05). The hemoglobin and hematocrit did not change in male and female fish in lidocaine treatment after both times. Cortisol changed in male and female in the sodium bicarbonate group after both times. The WBCs in females was significantly higher in clove extract and lidocaine treatments, whereas RBCs, Hb, Hct, and cortisol were significantly higher in males (P<0.05). Therefore, due to irreversible stressful effects of the sodium bicarbonate, it is not a suitable anesthetic for the studied fish. Although the stressful effects of lidocaine were lower than clove extract, especially in females, the clove extract has no irreversible effects and the stress-induced effects had been moderated 24 h after anesthesia. Hence, lidocaine and then clove extract is recommended as suitable anesthetics, especially for female Caspian kutum.

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Introduction

Anesthetics have significant applications in aquaculture such as sampling, transportation, artificial reproduction, hormone injections, vaccination, surgery, and biopsy (Ross and Ross, 2008; Lepic et al., 2014). In all above-mentioned applications, anesthetic agents decrease physiological activities, which reduce imposed stress and avoid illnesses and death (Keene et al., 1998). Nowadays, it seems necessary to explore anesthetic compounds with superior features such as rapid induction, easy and quick return, rapid disposal of tissues, and lack of toxicity at therapeutic levels, along with cheapness and availability. Hence, both natural and chemical compounds have been tested for this purpose (Hoseini et al., 2013; Mazandarani et al., 2015; Taheri Mirghaed et al., 2016; Mazandarani and Hoseini, 2016; Effati and Bahrekazemi, 2017; Yousefi et al., 2018). The commonly used anesthetics in aquaculture are tricaine methane sulfonate (MS222), benzocaine, quinaldine, metomidate, clove extract and 2-phenoxy ethanol (Lepic et al., 2014).

An active ingredient in clove extract and eugenol is 4-allyl-2-methoxyphenol (Ross and Ross, 2008). The effect of clove extract and clove oil has been studied on different fish species, including the roach (*Rutilus rutilus*), Caspian kutum (*R. kutum*), common carp (*Cyprinus carpio*), Persian sturgeon (*Acipenser persicus*), rainbow trout (*Oncorhynchus mykiss*), and beluga (*Huso huso*) (Wagner et al., 2002; Velisek et al., 2005; Soudagar et al., 2009; Imanpoor et al., 2010; Imanpoor and Farahi, 2011; Farahi et al., 2011; Hoseini, 2011; Hoseini and Ghelichpour, 2012; Mazandarani and Hoseini, 2017). Sodium bicarbonate ($NaHCO_3$) was first introduced in 1942 as an

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anesthetic in aquatic animals (Fish, 1942). By producing CO_2 from carbonic acid, sodium bicarbonate causes numbness in aquatic animals (Wagner et al., 2002). However, there are few studies on the anesthetic effect of this compound on fishes like rainbow trout, and common carp (Booke et al., 1978; Altun et al., 2009). Lidocaine with 2-(diethylamino)-N-(2, 6-dimethylphenyl) acetimide chemical compound, is insoluble in freebase water and it is generally used as a water-soluble hydrochloride salt form to anesthetize aquatic animals (Ackerman et al., 2005). Lidocaine has so far been used as an anesthetic in some species such as common carp, silver carp (*Hypophthalmichthys molitrix*), tilapia (*Oreochromis mossambica*), zebrafish (*Danio rerio*) and catfish (*Ictalurus punctatus*) (Carrasco et al., 1984; Ross and Ross, 2008; Collymore et al., 2014; Effati et al., 2014).

Although anesthetics can reduce physiological and biochemical stress in fish, some of these compounds may have side effects on hematological and biochemical parameters of blood. Accordingly, a side effect of such compounds on tensions in the aquatic species is expected (Lepic et al., 2014). Many studies have been conducted to describe physiological effects of various anesthetics in fishes; however, none of them compared physiological effects of the anesthetics and their differences in males and females. In addition, few researchers such as Imanpoor and Farahi (2011) have focused on the effects of three anesthetics in Caspian kutum. Therefore, this study aimed to investigate the effects of three anesthetics, namely clove extract, sodium bicarbonate, and lidocaine on the blood parameters and cortisol level in Caspian kutum and determine the best anesthetic in male and female broodstocks.

Materials and Methods

Experimental design: The experimental process was carried out in the Shirood River (Tonekabon, Mazandaran) in the southern Caspian Sea. During the Caspian kutum migration and their artificial reproduction in 2016, the broodstocks were collected. Among fish which were ready for propagation, 20 males and 20 females with age of 3 years, and average weight and total length of 352.5±56.2 g and 34.88±5.2 cm, respectively, were randomly selected.

Preparation of anesthetic solution: Lidocaine (2%) was obtained from the Pasteur Institute of Iran, sodium bicarbonate from the Soda Kaveh Chemical Industry Co. and clove extract from the Giah Essence Pharmaceutical Co. To prepare the anesthetic solutions, 50 mg/L clove extract, 150 mg/L lidocaine, and 300 mg/L sodium bicarbonate were added to a water tank and then, the resultant solution was completely stirred and homogenized (Table 1). After preparing water containing anesthetic solution with the desired concentrations (Ackerman et al., 2005; Velisek et al., 2005, 2011; Collymore et al., 2014), five male and five female broodstocks of each anesthetic group placed in it and the time of their introduction was registered. Fish were anesthetized through bathing in small tanks for starting the anesthetic process and then, they were transferred to larger tanks to recover (Velisek et al., 2006) (Table 1).

Blood sampling and blood parameters analyses: The blood samples were taken from the caudal vein in two steps (10 min and 24 h after recovery). For cortisol measurements, 2 ml of the blood was transferred to non-heparinized tubes, while the remaining blood (2 ml) was poured into the heparinized tubes for blood cell counts. The blood samples of the control fish with no anesthetics were also taken. The numbers of white blood cells (WBC) and red blood cells (RBC) were counted using a neubauer hemocytometer (Houston et al., 1996). Hematocrit was also measured by microhematocrit method (Rehulka, 2000), while hemoglobin was determined through the cyan-methaemoglobin method using spectrophotometer with 540 nm wavelength. (Blaxhall and Daisley, 1973). For serum preparation and cortisol assay, the blood was centrifuged at 3000 rpm for 10 min and the serum collected and kept frozen at -80°C. Cortisol level was determined using radioimmunoassay (Yaghobi et al., 2015).

Statistical analysis: The statistical model was designed as completely random which was performed by two way repeated ANOVA. The differences between treatments were investigated with Duncan's

Table 1. The doses of the anesthetics and the used procedure of anesthesia in this study.

Anesthetic	Dose	Anesthesia time (min)	Recovery time (min)
Clove extract	50 mg/L	5	5
Sodium bicarbonate	300 mg/L	5	10
Lidocaine	150 mg/L	2	5

Table 2. Blood and hormonal parameters measured in the male broodstocks of Caspian kutum under anesthesia treatments.

	RBC (N/mm^3)	WBC (N/mm^3)	Hb (g/dL)	Hct (%)	Cortisol (ng/mL)
Control	1183333±76376.26^{abc}	8833.33±152.75^{a}	9.37 ±0.31^{ab}	27.67±0.58^{ab}	313.00±9.89^{a}
Clove extract (10 min.)	1133333±28867.51^{ab}	8266.67±1193.03^{a}	12.87 ±0.21^{c}	39 .00±1^{c}	418.67±87.55^{a}
Clove extract (24 h)	1140000±52915.03^{abc}	7166.67±461.88^{a}	11.93±0.38^{bc}	37.67 ±0.58^{c}	422.67±67.53^{ab}
Lidocaine (10 min.)	1200000±111355.29^{abc}	7566.67±503.32^{a}	11.5±0.75^{bc}	33.63±1.53^{bc}	437.67±22.68^{ab}
Lidocaine (24 h)	936666±49328.83^{a*}	6066.67±404.14^{a*}	9.63±0.15^{ab*}	20.33±1.53^{a*}	347.33±24.58^{a}
Sodium bicarbonate (10 min)	1323333±256969.52^{bc}	13533.33±3780.6^{b}	11.33±2.71^{bc}	34.33±7.37^{bc}	700.00±50.91^{c}
Sodium bicarbonate (24 h)	1463333±327159.49^{c}	14800±3064.31^{b}	12.5±3.24 ^{c}	40±11.27^{c*}	530.79±55.69^{b*}

Data are presented as means ± standard error. Significant differences of the blood parameter between different anesthetics at two sampling times and the control group are indicated by unlike letters in each column (P<0.05; Duncan's test), and significant differences of blood parameter between 10 min and 24 h sampling for each anesthetic are shown by the asterisk (*) (P<0.05; t-test).

test. The Pairwise t-test was also employed to compare blood parameters and cortisol of the sampling times in 10 min and 24 h after anesthesia. Pillai's multivariable analyze was used to compare the number of RBCs and WBCs along with the values of cortisol, hemoglobin and hematocrit in male and female broodstocks. All analysis were done by SPSS (version 23) at a confidence level of 95%. All values were presented as Mean±SD.

Results

Male broodstocks: The results showed that 10 min after anesthesia, number of RBCs in the control group was almost the same as other three treatments. 24 hours after anesthesia, a little decrease of RBCs was observed in the groups of clove extract and lidocaine with a little increase of RBCs in the sodium bicarbonate group. However, differences were not significant with that of the control group (P>0.05) (Table 2). Comparison of 10 min and 24 h after anesthesia for RBCs counts in males revealed significant differences. Since the WBCs in the control male fish was 8833.33±152.7 in mm^3, the only different group was those anesthetized with sodium bicarbonate (P<0.05), 10 min and 24 h after anesthesia (Table 2). In addition, comparison of WBCs in male broodstocks showed significant differences only with lidocaine (P<0.05) (Table 2).

Hemoglobin levels in male of the control group was estimated as 9.37±0.31 g/dL and it is increased in all anesthetics group, 10 min after anesthesia with a significant increase in clove extract (P<0.05). 24 h after anesthesia, the fish anesthetized with sodium bicarbonate were only group having significant increase (Table 2). Comparison of the hemoglobin in males revealed significant differences in 10 min and 24 h in lidocaine treatment (P<0.05) (Table 2). Moreover, the hematocrit in males of the control group was 27.67±0.58%, and it was significantly elevated in the group with clove extract in 10 min after anesthesia (P<0.05) and comparing to control group, the hematocrit levels were increased in male fish anesthetized with sodium bicarbonate and clove extract after 24 h (Table 2). The results showed that the hematocrit in males anesthetized with clove extract at 10 min and 24 h after anesthesia was almost the same (P>0.05) (Table 2).

The cortisol levels in male fish of the control group was 313.00±9.89 ng/mL. Although levels of the hormone elevated after anesthesia in all treatments, significant differences were observed only in sodium bicarbonate treatment in both times of the sampling (P<0.05) (Table 2). Comparisons of the cortisol level in male showed the same result (P<0.05) (Table 2).

Female broodstocks: The number of RBCs in control female broodstocks was significantly different from

Table 3. Blood and hormonal parameters in the female broodstocks of Caspian kutum under anesthesia treatments.

	RBC (N/mm^3)	WBC (N/mm^3)	Hb (g/dL)	Hct (%)	Cortisol(ng/mL)
Control	968333.3±75883.68^{a}	7333.33±207.26^{a}	7.3±0.46 ^{a}	23.33±0.58 ^{a}	325.50±31.82 ^{a}
Clove extract (10 min.)	1393333±187705.44^{b}	12300±468.22^{ab}	12.93±1.65 ^{c}	39.67±5.51 ^{c}	383.50±12.16 ^{ab}
Clove extract (24 h)	993333.3±66583.28^{a*}	9166.67±445.09^{ab}	8.47±0.49 ^{ab*}	25.67±1.53 ^{a*}	343.33±11.42 ^{ab}
Lidocaine (10 min)	966666.7±15275.25^{a}	8066.67±378.59^{ab}	6.5±0.2 ^{a}	22.67±2.52 ^{a}	361.00±39.59 ^{ab}
Lidocaine (24 h)	1026667±49328.28^{a}	7450±353.55^{a*}	8.57±0.3 ^{ab*}	26±1 ^{a}	302.33±41.64 ^{a*}
Sodium bicarbonate (10 min)	1413333±55075.7^{b}	12900±100^{b}	13.03±0.21 ^{c}	40.33±1.53 ^{b}	637.00±24.88 ^{c}
Sodium bicarbonate (24 h)	1403333±50332.23^{b}	9433.33±602.77^{ab}	10.47±2.76 ^{b*}	40±1 ^{b}	490.00±20.52 ^{b*}

Data are presented as means ± standard error. Significant differences of the blood parameter between different anesthetics at two sampling times and the control group are indicated by unlike letters in each column (P<0.05; Duncan's test), and significant differences of blood parameter between 10 min and 24 h sampling for each anesthetic are shown by the asterisk (*) (P<0.05; t-test).

Table 4. Multivariate Pillai test results of the blood and hormonal parameters.

Effect	Formula
Red blood cell	
Sampling time (10 min and 24 h)	Pillai=0.002, F (1,19)= 0.031, Sig= 0.862
Sampling time × Anesthetic drug	Pillai=0.332, F (3,19)= 3.15, Sig= 0.049
Gender (Male and Female)	Pillai=0.028, F (1,19)= 0.555, Sig= 0.465
Hemoglobin	
Sampling time (10 min and 24 h)	Pillai=0.013, F (1,19)= 0.248, Sig= 0.624
Sampling time × Anesthetic drug	Pillai=0.328, F (3,19)= 3.088, Sig= 0.052
Gender (Male and Female)	Pillai=0.058, F (1,19)= 1.161, Sig= 0.295
Hematocrit	
Sampling time (10 min and 24 h)	Pillai=0.006, F (1,19)= 0.106, Sig= 0.748
Sampling time × Anesthetic drug	Pillai=0.360, F (3,19)= 3.563, Sig= 0.034
Gender (Male and Female)	Pillai=0.064, F (1,19)= 1.298, Sig= 0.269
White blood cell	
Sampling time (10 min and 24 h)	Pillai=0.473, F (1,19)= 17.03, Sig= 0.001
Sampling time × Anesthetic drug	Pillai=0.422, F (3,19)= 4.62, Sig= 0.014
Gender (Male and Female)	Pillai=0.383, F (1,19)= 11.811, Sig= 0.003
Cortisol	
Sampling time (10 min and 24 h)	Pillai=0.928, F (1,19)= 246.099, Sig= 0.00
Sampling time × Anesthetic drug	Pillai=0.909, F (3,19)= 63.417, Sig= 0.00
Gender (Male and Female)	Pillai=0.037, F (1,19)= 0.735, Sig= 0.402

those in treated groups with clove extract and sodium bicarbonate 10 min after anesthesia (P<0.05). Comparing to the control group, the RBCs 24 h after anesthesia was markedly increased in female fish received sodium bicarbonate (Table 3). Comparisons of the RBCs count in females revealed a significant differences 10 min and 24 h after anesthesia only in clove extract treatment (P<0.05) (Table 3).

The WBCs in control female fish was 7333.33±207.26 in mm^3; the females anesthetized with all three anesthetics exhibited incremental trends, which was significant in fish treated with sodium bicarbonate (P<0.05). Comparing to the control group, the WBCs in all three anesthetic treatments was not significantly different 24 h after anesthesia (P>0.05) (Table 3). Comparisons of the WBCs in female broodstocks confirm significant differences only in lidocaine treatment in 10 min and 24 h (Table 3).

Hemoglobin levels in the females of the control group was 7.3±0.46 g/dL, which significantly increased 10 min after anesthesia with clove extract and sodium bicarbonate (P<0.05). Compared to the control group, a significant increases observed 24 h after anesthesia in sodium bicarbonate group (Table 3). Considerable differences after 10 min and 24 h after anesthesia are observed in all treatments (P<0.05) (Table 3). Also, the hematocrit in females of the control group was 23.33±0.58% and increased 10 min after anesthesia with clove extract and sodium bicarbonate (P<0.05). The hematocrit levels markedly

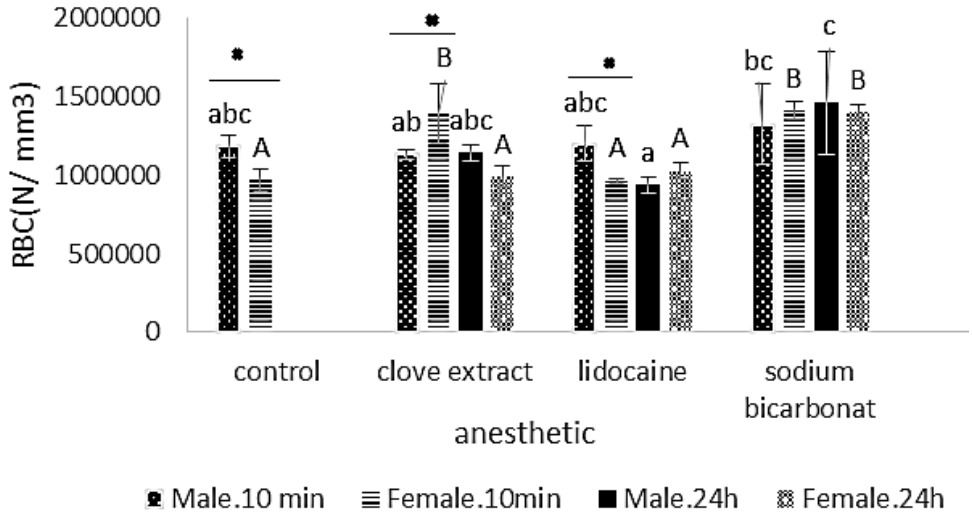

Figure 1. Effect of anesthesia by clove extract, lidocaine, and sodium bicarbonate on RBCs count of male and female broodstocks in 10 min and 24 h after anesthesia. Different lowercase letters show significant difference between RBCs count in the males in 10 min and 24 h sampling times with the control group and each other; whereas, different uppercase letters show significant difference between RBCs count in the females in 10 min and 24 h sampling times with the control group and each other. Asterisks show significant difference between the males and females' RBCs count in each treatment.

increased in females 24 h after anesthesia with sodium bicarbonate (Table 3). Comparisons of the hematocrit in female broodstocks anesthetized with clove extract resulted in significant differences between 10 min and 24 h post-anesthesia (P<0.05) (Table 3).

The serum cortisol level in female fish of the control group was 325.50±31.82 ng/mL. 10 min and 24 h after anesthesia by sodium bicarbonate, the levels of the hormone were elevated significantly (Table 3). The results also showed a significant differences in the cortisol levels in the broodstocks at 10 min and 24 h after anesthesia with sodium bicarbonate (Table 3).

Comparative results of Pillai multivariable analyze: Multivariate Pillai test of the red blood cells showed no differences in 10 min and 24 h after anesthesia. However, the mean number of RBCs was different in two sampling times of 10 min and 24 h and various types of anesthetic agents. Also, the mean number of the red blood cells was not significantly different between male and female in two sampling times (P>0.05) (Table 4). Applying multivariate Pillai test on the hemoglobin and hematocrit showed similar results of the RBCs. Despite significant differences between their mean values in two sampling times and three anesthetic drugs, no difference was observed between male and female (P>0.05) (Table 4). However, the results on the WBCs were different from other parameters. The mean number of the white blood cells in terms of time after anesthesia, type of the anesthetic drugs, and gender was different significantly (P<0.05) (Table 4). Furthermore, a significant differences were observed between the mean value of the cortisol in terms of time after anesthesia and type of anesthetic drugs (P<0.05). However, the amount of cortisol based on the gender was almost unchanged in two sampling time (P>0.05) (Table 4).

The results between male and female broodstocks showed a significant difference between the RBCs in the control group and clove extract and lidocaine treatments only at 10 min, so that only in clove extract, the RBCs in females was higher than that of males (P<0.05) (Fig. 1). Furthermore, the differences in the WBCs was observed between control group and clove extract treatment at 10 min and 24 h after anesthesia, and the lidocaine and sodium bicarbonate treatments only in 24 h after anesthesia (P<0.05). The WBCs of females was lower in the control group and sodium bicarbonate, while the WBCs of males was lower in the clove extract and lidocaine (Fig. 2). In the hemoglobin, the difference between male and female broodstocks was significant in the control group and clove extract at 24 h and lidocaine at 10 min and the hemoglobin in both anesthetics was lower in females

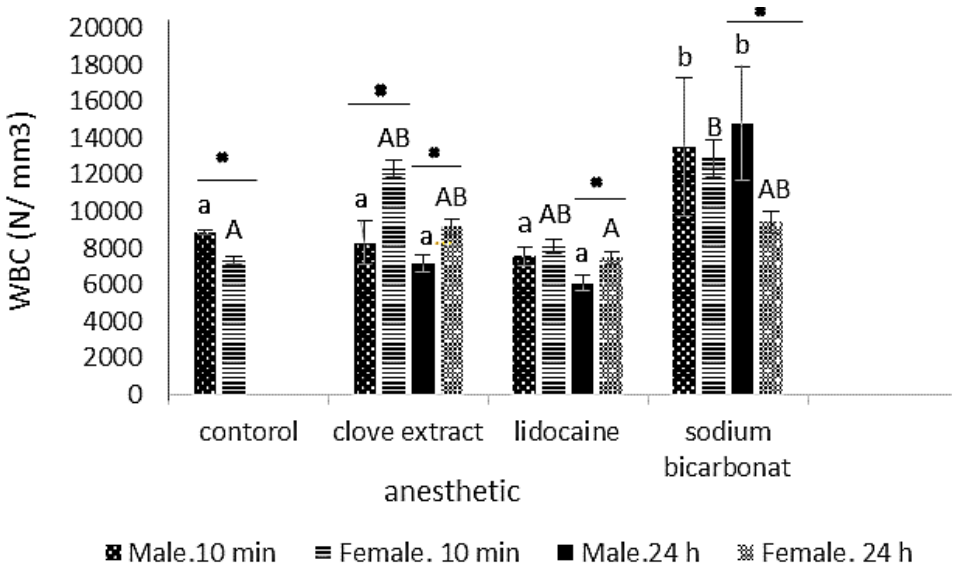

Figure 2. Effect of anesthesia by clove extract, lidocaine, and sodium bicarbonate on WBCs count of male and female broodstocks in 10 min and 24 h after anesthesia. Different lowercase letters show significant difference between WBCs count in the males in 10 min and 24 h sampling times with the control group and each other; whereas, different uppercase letters show significant difference between WBCs count in the females in 10 min and 24 h sampling times with the control group and each other. Asterisks show significant difference between the males and females' WBCs count in each treatment.

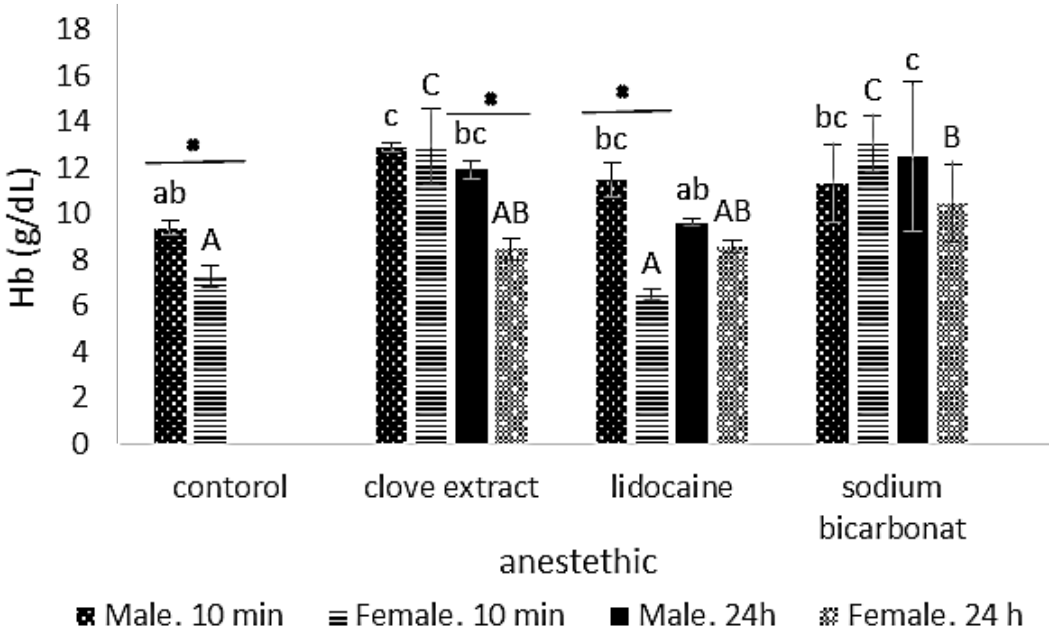

Figure 3. Effect of the anesthesia by clove extract, lidocaine, and sodium bicarbonate on Hb amount of male and female broodstocks in 10 min and 24 h after anesthesia. Different lowercase letters show significant difference between Hb amount in the males in 10 min and 24 h sampling times with the control group and each other; whereas, different uppercase letters show significant difference between Hb amount in the females in 10 min and 24 h sampling times with the control group and each other. Asterisks show significant difference between the males and females' Hb amount in each treatment.

(P<0.05) (Fig. 3). In terms of the hematocrit, there was a significant difference between male and female in the control group, and clove extract in 24 h, bicarbonate sodium in 10 min, and lidocaine in both sampling times, so that the Hct was higher in females in lidocaine in 24 h and sodium bicarbonate in 10 min (P<0.05) (Fig. 4). However, the results of the cortisol were different since significant differences were observed between male and female broodstocks in clove extract in 24 h, sodium bicarbonate in 10 min, and lidocaine in both times. Moreover, the hormone was lower in females in all three anesthetic groups

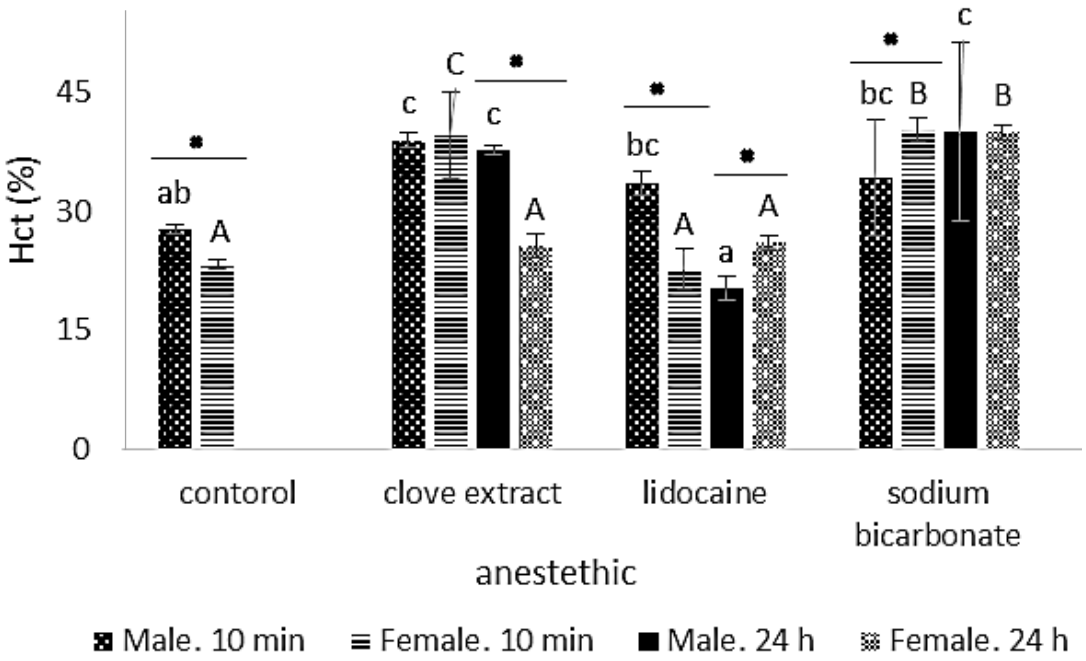

Figure 4. Effect of the anesthesia by clove extract, lidocaine, and sodium bicarbonate on Hct amount of male and female broodstocks in 10 min and 24 h after anesthesia. Different lowercase letters show significant difference between Hct amount in the males in 10 min and 24 h sampling times with the control group and each other; whereas, different uppercase letters show significant difference between Hct amount in the females in 10 min and 24 h sampling times with the control group and each other. Asterisks show significant difference between the males and females' Hct amount in each treatment.

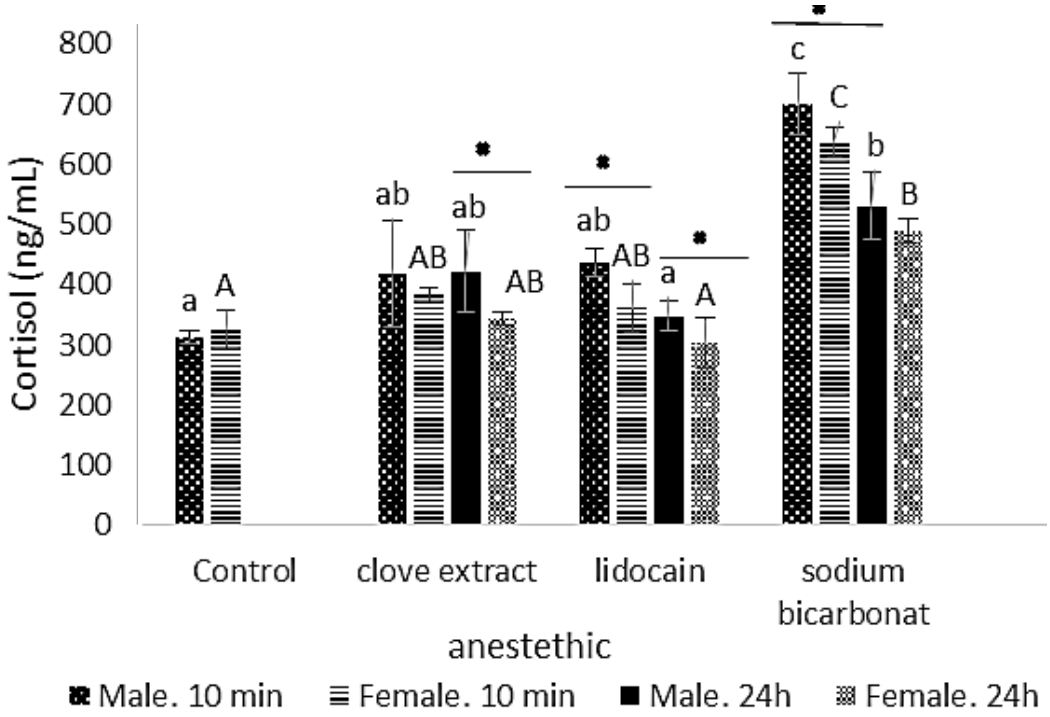

Figure 5. Effect of the anesthesia by clove extract, lidocaine, and sodium bicarbonate on cortisol amount of male and female broodstocks in 10 min and 24 h after anesthesia. Different lowercase letters show significant difference between cortisol amount in the males in 10 min and 24 h sampling times with the control group and each other; whereas, different uppercase letters show significant difference between cortisol amount in the females in 10 min and 24 h sampling times with the control group and each other. Asterisks show significant difference between the males and females' cortisol amount in each treatment.

(P<0.05) (Fig. 5).

Discussions

Since different species of fish show different responses to anesthetic substances, determination of the type and dosage for any species is necessary (Lepic et al., 2014). Analysis of the blood parameters is one of the most important and reliable methods that can provide important information about healthy conditions of an organisms (Kristan et al., 2012). There is a close relationship between changes of RBCs, hemoglobin, and hematocrit and the stressful environmental factors. Releasing of the cathecolamines are primary stress response causing erythrocytes to swell and spleen releases new erythrocyte to blood. This will consequently lead to increase of Hct and RBCs as well as hemoglobin levels. All these changes lead to increase of oxygen carrying capacity of the blood to supply demanded oxygen under stressful conditions (Wendelaar Bonga, 1997). Therefore, increasing the number of RBCs indicates high stress levels in fish (Wedemeyer et al., 1990). In this study, no significant differences were observed between the experimental treatments and control group in the RBCs in male fish, 10 min and 24 h after anesthesia. For females, however, anesthetized fish with clove extract and sodium bicarbonate were significantly different in 10 min after anesthesia. 24 h after anesthesia, the RBCs of females anesthetized with sodium bicarbonate was the only case with significant increase. In addition, according to the anesthetic type, there was a considerable difference in the red blood cells in two sampling times as well as Hb and Hct. Also, by comparing males and females, significant increase appears in the RBCs of females in the group of clove extract treatment 10 min after anesthesia. The dissimilar results between two genders may be due to differences in their physiology (Farrell et al., 2011). Imanpoor and Farahi (2011) showed that using clove extract for anesthetizing male broodstocks of the Caspian kutum had no significant effects on their RBC. Velisek et al. (2006) worked on the catfish (*Silurus glanis*) anesthetized with clove oil, showing that the RBCs were not significantly different in 10 min and 24 h after anesthesia. Similar results were obtained by Soudagar et al. (2009) on the roach (*R. rutilus*), Farahi et al. (2011) on the adult males of Caspian kutum, Effati and Bahrekazemi (2017) on grass carp (*Ctenopharyngodon idella*), Effati et al. (2014) on silver carp, and Lepic et al. (2014) on vimba (*Vimba vimba*), all of which are consistent with our results in male broodstocks but not with those found in female fish. In addition, like clove extract after 10 min, the use of clove oil in female Persian sturgeon caused a significant increase in blood parameters (RBCs, Hb, and Hct) (Mazandarani et al., 2015).

The present study showed that 10 minutes after anesthesia, the hemoglobin was elevated in all treatments with a significant increase in the males and females in the clove extract treatment and female in the sodium bicarbonate treatment. 24 h after anesthesia, significant increase was observed in male and female of the sodium bicarbonate treatments. According to high levels of hemoglobin in the males and females anesthetized with sodium bicarbonate even 24 h after anesthesia, it can be concluded that stressful conditions remained after that period. Velisek et al. (2005, 2006) used clove oil, not clove extract, to anesthetize common carp and catfish and concluded no significant effects on hemoglobin. The results showed a significant reduction of the hemoglobin in the female broodstocks in clove extract and lidocaine treatments. RBC, Hb and Hct for female broodstocks were lower than males even in the control group.

In addition, the hematocrit significantly increased 10 min and 24 h after anesthesia with clove extract and sodium bicarbonate treatments. A study by Chen (2012) showed that after anesthesia, the Hct of goldfish (*Carassius auratus*) was significantly increased by lidocaine treatment, which was in contrast to our results that could be due to difference in species type and dosage of lidocaine, as well as the physicochemical conditions of the experimental water. Despite lower Hct in clove extract treatment in females, in the two other anesthetics, the Hct was higher in female.

Although Roos and Roos (2008) deduced that stress may increase the number of white blood cells, the fact is that WBCs may change during stress but the effect of stress on WBCs is inconsistent. The brain and body of fish have constant communication with each other. During acute or short-term stress, the first response of the brain is to help the body deal with the situation, which might increase number of WBCs. At the end of stressful event, the brain sends signals to reverse the initial stress reaction, allowing the WBCs to return to normal situation (Roos and Roos, 2008). In the present study, although the WBCs increased in 10 min sampling, it was significant only in sodium bicarbonate treatment in both genders. In addition, the number of WBCs was decreased 24 h after anesthesia in all treatments. Our results are in agreement with above-mentioned arguments. Therefore, by relieving anesthesia stress after 24 h, WBCs were decreased in all treatments. Furthermore, the number of WBCs was significantly different between anesthetics in two sampling times and gender with lower amounts in females only in sodium bicarbonate. It is concluded that stress-inducing effect of sodium bicarbonate in females is lower. A study by Farahi et al. (2011) on male *C. gibelio* anesthetized with clove extract yielded no significant changes in WBC counts, which is similar to the results of the present study in male fish anesthetized with clove extract. In addition, Imanpoor and Farahi (2011) showed that using clove extract for anesthetizing male Caspian kutum fish no effect on WBCs. However, Soudagar et al. (2009) in roach and Imanpoor et al. (2010) in Persian sturgeon reported that the number of WBCs has been elevated 10 min after anesthesia with clove extract, which was not significant 24 h after anesthesia. This result is in contrast to our finding. Among possible reasons for these dissimilar results, species, age, gender, and condition of rearing condition can be mentioned (Ross and Ross, 2008).

Cortisol is the most important indicator of the stress (Wagner et al., 2002). In the present study, the cortisol showed significant differences in the sodium bicarbonate treatment in male and female at both sampling times. There were also significant differences based on the sampling time and gender. The cortisol levels in females were lower, which was significant only in the lidocaine group at both times. The stress-induced effect of the sodium bicarbonate in both sexes is greater than those of the clove extract and lidocaine, in which stress levels in females were less than males. Wagner et al. (2002) reported that serum cortisol levels decreased in rainbow trout treated with clove extract indicating diminished stress levels. Clove extract may block the transmission of sensory information to the hypothalamus and prevent activation of the hypothalamus-pituitary-interrenal axis. In this regard, Small (2003) studied the effects of clove extract on the channel catfish, showing high efficiency of the clove extract in suppressing the secretion of cortisol, which is in agreement with our results especially 24 h after anesthesia. Effati and Bahrekazemi (2017) reported that cortisol level was significantly higher in 10 min and 24 h times after lidocaine and sodium bicarbonate treatments comparing to the control group in grass carp which is similar to sodium bicarbonate results in the Caspian kutum. In Persian sturgeon, the non-anesthetized female fish had significantly higher cortisol levels than those anesthetized with clove oil (Mazandarani et al., 2015) which is in contrast to our finding. The reason might be large size of the Persian sturgeon.

Conclusion

According to the blood parameters, especially cortisol levels, sodium bicarbonate was not an appropriate anesthetic for male and female broodstocks since stressful effects were continued up to 24 h after induction of anesthesia. Comparing other two anesthetics, i.e. clove extract and lidocaine, the effects of the lidocaine were lower than clove extract especially in females. However, the latter has no irreversible effects, and after 24 h of anesthesia, stress-induced effects had been greatly moderated. Therefore, lidocaine and then clove extract can be recommended as suitable anesthetics, especially in female Caspian kutum.

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